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Is there an ideal animal model for SARS?

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The outbreak of severe acute respiratory syndrome (SARS) in 2003 was controlled by public health measures at a time when specific interventions such as antiviral drugs, vaccines and immunotherapy were not available. Since then, several animal models have been developed for the study of SARS and, although no model replicates the human disease in all aspects, the use of animal models for SARS has led to the establishment of several important principles for vaccine and immunotherapy. Consistency and reproducibility of findings in a given model must be demonstrated to establish the superiority of one model over others. Here, we suggest aspects of an ideal animal model for studies of SARS pathogenesis and vaccine development and present our assessment of the strengths and limitations of the current animal models for SARS.

Emergence and identification of SARS-CoV: a new human pathogen

In early 2003, an outbreak of severe respiratory disease that first came to the attention of the public health authorities in Hong Kong rapidly spread to Canada, Singapore and Vietnam by returned travelers. In a few weeks, >8000 cases and 774 deaths due to severe acute respiratory syndrome (SARS) were reported in 30 countries (http://www.who.int/csr/sars/country/table2004_04_21/en/index.html). The outbreak was controlled with public health measures that included use of a broad case definition, hospitalization and isolation of suspect cases, quarantine and travel advisories.

The etiological agent of SARS is a novel coronavirus (CoV) referred to as SARS-CoV [1–4], which was introduced into humans from an animal reservoir. Viruses that are closely related to SARS-CoV have been isolated from civet cats [5] and horseshoe bats [6,7] but the source of SARS-CoV has not been definitively identified.

Since the large global outbreak of 2003, a limited number of cases of SARS have occurred that included four community-acquired infections associated with mild disease the following winter, and at least four laboratory-acquired infections, one of which resulted in secondary spread and severe illness and mortality in contacts. Although it is difficult to predict whether another

outbreak of SARS will occur, exposure to infected animals and laboratory-acquired infections that are not immediately recognized are two potential sources for infections. The high morbidity and mortality rate and economic consequences of the 2003 outbreak, together with the potential for infections in the future, are reasons for continued global interest in the development of measures to prevent and control SARS.

Antiviral drugs, immunotherapy and vaccines against SARS will have to be evaluated in animal models, and a rational selection of an appropriate model for these studies will depend on a careful assessment and review of available models. We summarize the findings in the current animal models for SARS and discuss what we have learned from these models. The utility, benefits, limitations and controversy surrounding these models are also discussed. Furthermore, we suggest directions for future research that will facilitate the resolution of controversial findings, and we present some of the outstanding research questions in the field.

The development of animal models for SARS

The international cooperation and coordination that led to the rapid identification of SARS-CoV and the control of the SARS outbreak were exemplary. Efforts to develop animal models were undertaken in several laboratories around the world and experimental infection in these different animal models has proven to be invaluable in establishing the basis for prevention and control strategies for SARS. However, published reports from laboratories that have used different animal models have led to some controversial opinions and misunderstandings about animal models for SARS. It is essential that the findings in a given model are consistent and reproducible and that these features are well documented.

The use of different animal models can be tailored to the goals of each study. Consistency, reproducibility and the relevance of the outcome measures to the scientific question (such as immunogenicity and efficacy of vaccines or pathogenesis of disease) must be considered before one can conclude that a given model is superior to other available models and is the preferred or appropriate model. Clearly, the pathogenesis of disease can only be studied in animal models that replicate key aspects of the disease. But animal models do not need to replicate all aspects of disease to provide useful

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Available online 8 June 2006

information in evaluation of vaccines and immunoprophylaxis. For example, an animal model can be useful in vaccine efficacy studies if the following two criteria are met: (i) virus titer correlates with disease severity; and (ii) the virus replicates in the animal model to a sufficiently high titer so that differences in the level of replication between immunized and mock-immunized control groups can be meaningfully distinguished. Under these conditions, demonstration that a vaccine or antibody can prevent or significantly reduce the level of replication of challenge virus would be convincing evidence of efficacy. There is ample precedence for this approach in the development of vaccines against other respiratory viruses.

There is a widespread but unsubstantiated belief that ferrets and nonhuman primates are the only acceptable models for the study of SARS. Based on our experience studying SARS-CoV infections in several species (including mice, hamsters and three species of nonhuman primates), and after a careful review of published reports, we would argue that there are pros and cons for each of the animal models and that the choice of the model used can be guided by the nature of the study (Table 1). Not surprisingly, the most reproducible data on virus replication is obtained in inbred mice and in golden Syrian hamsters that are not inbred but are of limited genetic heterogeneity.

Mouse models

Mouse species that have been infected with SARS-CoV include BALB/c [8,9], C57BL6 (B6) [10], 129S Sv/Ev and STAT 1 knockout mice [11]. When SARS-CoV is administered intranasally to lightly anesthetized 4–8-week-old BALB/c or B6 mice, the virus replicates to high titer in the upper and lower respiratory tract (nasal turbinates and lung tissues, respectively) without associated signs of morbidity or mortality [8,10]. Virus replication in the respiratory tract peaks at day two or three post-infection (pi) but it is not accompanied by substantial pulmonary inflammation or pneumonitis. The virus is cleared from the lungs by day five to seven pi [8,10]. Transient weight loss and pneumonitis are seen in strain 129S mice following SARS-CoV infection [11].

Old (12–14-month-old) BALB/c mice develop signs of clinical illness (weight loss, ruffled fur and mild dehydration) and histopathological evidence of disease (bronchiolitis, patchy interstitial pneumonitis and diffuse alveolar damage) following SARS-CoV infection; this age-related increase in morbidity in BALB/c mice is reminiscent of observations in humans in the 2003 SARS outbreak in which age was a significant risk factor for severe disease and poor outcome [12].

Because SARS-CoV replicates to high titer in the respiratory tract of mice and findings in mice are highly reproducible, the mouse model can be used for evaluation of vaccines, immunoprophylaxis and antiviral

Table 1. Animal species that have been experimentally infected with SARS-CoV

Animal species	Virus strain (route of inoculation)	Main findings	Proposed application	Limitations	Refs
Young inbred mice	Urbani (IN) Urbani (IN, PO)	Viral replication	Vaccines, antivirals	No illness	[8–10]
Old BALB/c mice	Urbani (IN)	Viral replication associated with signs of clinical illness, interstitial pneumonitis and diffuse alveolar damage	Pathogenesis, vaccines, immunoprophylaxis	Availability, immune senescence	[12]
STAT 1 ^{-/-} mice	Tor2 (IN) Urbani (IN)	Viral replication, morbidity and mortality and pneumonitis	Antivirals, pathogenesis	Defect in innate immunity	[11]; K. Subbarao and A. Roberts, unpublished
Ferrets	HKU-39849 (IT) Tor2 (IN)	Viral replication and pneumonitis	Vaccines, immunoprophylaxis, immunotherapy, antivirals	Needs further characterization; availability, susceptibility to other respiratory viruses	[26,28]
Hamsters	Urbani (IN) Frankfurt (IN) HKU-39849 (IN)	Viral replication and interstitial pneumonitis, consolidation and diffuse alveolar damage	Vaccines, immunoprophylaxis, immunotherapy, antivirals	No illness or overt disease, lack of immunological reagents	[24]; K. Subbarao and A. Roberts, unpublished
Old World primates: cynomolgus, African green and rhesus monkeys	HKU-39849 (IT) ^b Urbani (IN, IT) Tor2 (IT, IV) PUMC01 (IN)	Viral replication and pneumonitis (diffuse alveolar damage)	Immunogenicity of vaccines, immunoprophylaxis, antivirals	Availability, cost, housing, statistical analysis limited	[29–33]
New World primates: common marmosets	Urbani (IT)	Pneumonitis, diarrhea and hepatitis	Pathogenesis, immunogenicity of vaccines, immunoprophylaxis, antivirals	Availability, cost, housing, statistical analysis limited, infectious virus was not recovered	[34]

^aAbbreviations: IN, intranasal; IT, intratracheal; PO, oral.

^bThe virus used in this study was identified as HKU-39849 by Peiris *et al.* [37].

drugs [8,13–23]. Additionally, the available range of immunologic reagents and knockout mice make it possible to carry out studies of pathogenesis in mice that develop pneumonitis in association with viral replication.

Hamster model

Golden Syrian hamsters are excellent models for SARS-CoV infection [24] because the virus replicates to high titer in the respiratory tract [10^8 50% tissue culture infectious doses (TCID₅₀) per gram of lung tissue following intranasal administration of 10^3 TCID₅₀ of virus] with associated interstitial pneumonitis, pulmonary consolidation and diffuse alveolar damage. Infection is not accompanied by overt clinical illness and the virus is cleared seven to ten days pi. As in mice, SARS-CoV infection of golden Syrian hamsters elicits a robust neutralizing antibody response and previously infected hamsters are protected from subsequent infection [24]. A correlation between the level of virus recovered from the lungs and the extent of pneumonitis has been demonstrated in SARS-CoV infected hamsters [25]. The fact that SARS-CoV reproducibly replicates to extremely high titer in the respiratory tract of hamsters with associated pneumonitis makes this an excellent model for the evaluation of vaccines, immunoprophylaxis and immunotherapy for SARS [24,25].

Ferret model

Ferrets and domestic cats were found to be susceptible to infection with SARS-CoV [26] but further studies in domestic cats have not been reported. The virus replicates in the lungs following intranasal infection [27,28] and is reported to be associated with pneumonitis but there are some conflicting unpublished reports of the presence, nature and severity of clinical findings in ferrets. Photomicrographs of the evolution of histopathological findings following primary infection with SARS-CoV in ferrets have not been published so it is not yet possible to compare the findings in ferrets with those in other animal models. Therefore, based on the evaluation of published reports, it would be premature to conclude that ferrets are a superior model for SARS.

Non-human primate models

Several species of Old and New World primates were evaluated as models for SARS. When virus was administered into the respiratory tract, infections in cynomolgus [29–31] and rhesus macaques [31–33], African green monkeys [31] and common marmosets [34] were reported with variable degrees of associated clinical illness and disease, alongside variable success in the isolation of infectious virus from tissues. Attempts to experimentally infect squirrel monkeys and mustached tamarins were not successful (K. Subbarao and A. Roberts, unpublished). Virus replication was detected in the respiratory tract of cynomolgus and rhesus macaques and African green monkeys, and there is at least one report of pneumonitis in each of these species [30–33]. Fever, watery diarrhea, pneumonitis and hepatitis were observed in common marmosets [34].

Only one group of investigators have each studied SARS-CoV infection in African green monkeys and common marmosets [31,34], whereas two or more groups of investigators have evaluated cynomolgus [30,31,33] and rhesus macaques [31–33]. Unfortunately, reports on clinical illness in cynomolgus and rhesus macaques from the various groups are contradictory. Clearly there are several factors that can contribute to the differences in findings by different investigators, including age and source of the animals, their environmental history and possible presence of co-pathogens, and the dose, route of inoculation and strain of virus administered. However, when findings are not reproducible in outbred species that demonstrate biological variability, it becomes particularly important that studies are carried out in large sample sizes to draw meaningful conclusions. The difficulty in conducting studies in non-human primates with large sample size limits their utility as an ideal animal model.

What have we learned from SARS animal models?

We have learned much about correlates of immunity using the current animal models for SARS research. Experiments in mice with targeted defects in the immune system suggest that innate immunity has an important role in clearance of SARS-CoV [10,11]. Additional studies have shown that intranasally administered SARS-CoV replicates in the respiratory tract of several animal species and protects animals from re-infection with SARS-CoV [8,24,35]. In addition to primary infection, active

Table 2. Passive and active immunization strategies against SARS-CoV evaluated in animal models

Type of immunization	Species Mice	Ferrets	Hamsters	Non-human primates
Passive				
Monoclonal antibodies	[15,21,22]	[27]	[25]	ND
Polyclonal sera	[8]	ND	K. Subbarao and A. Roberts, unpublished	ND
Active				
Subunit	[14]	ND	Y.W. Kam <i>et al.</i> , unpublished	ND
Inactivated	[17,18,20]	ND	ND	[38]
DNA	[19,23]	ND	ND	ND
Vectored	[13,16,18]	[28]	[35]	[36,39]

^aAbbreviation: ND, not determined.

Box 1. Limitations of the available animal models

- The kinetics of replication are more rapid in animal models than in humans. This observation could lead to an overestimation of the importance of innate immunity over adaptive immunity in clearance of SARS-CoV and the importance of antibodies in providing protection.
- Some findings, particularly clinical observations, are not reproducible in most models.
- Attempts to recover infectious virus from tissues are not always successful.
- None of the available models fully replicate the severe disease seen in humans.

immunization with inactivated whole virus vaccines, DNA vaccines, subunit vaccines and live, vectored vaccines expressing the SARS-CoV spike protein also elicit neutralizing antibodies to SARS-CoV that provide protection from subsequent challenge (Table 2). Furthermore, passive transfer of post-infection or post-immunization sera or monoclonal antibodies against the spike protein can also protect mice, ferrets and hamsters from infection, and modulate disease (Table 2). Moreover, depletion of T-cells in mice immunized with a DNA vaccine encoding the spike protein gene did not abrogate protection from SARS-CoV challenge, and adoptive transfer of T-cells from immunized mice to naive mice did not confer protection from SARS-CoV challenge. These findings strongly suggest that vaccines that elicit humoral immunity to SARS-CoV spike protein or immunotherapy that targets the spike protein will be sufficient to protect from SARS infection and associated disease.

Research in animal models has led to the identification of correlates of immunity such as the protective ability of actively induced neutralizing antibodies [8,13,14,16,17,23,35,36] or passively transferred neutralizing antibodies [8,15,21,22]. These principles will provide the basis for prevention and intervention strategies in the event that SARS-CoV reappears in humans.

Is there an ideal animal model for SARS?

In conclusion, the development of animal models for SARS has progressed rapidly; the described models range from those in which only virus replication is observed (such as young BALB/c or B6 mice) to models in which virus replication is accompanied by histopathologic evidence of disease (such as hamsters, ferrets, African green monkeys, cynomolgus and rhesus macaques) and those in which consistent findings of clinical illness and histopathological evidence of disease are observed (old BALB/c mice). Although much has been learned since the initial description of the various animal models and from subsequent studies in which vaccines or therapeutic or prophylaxis strategies were studied in different animal models, the limitations of the various models (Box 1) must also be recognized. Available data do not yet support a conclusion that there is a single preferred model for SARS in which all intervention or preventive strategies must be evaluated.

Outstanding questions

Further research should be undertaken in existing and new animal models to address some of the outstanding questions listed below.

- How well can prior infection or vaccines protect animals from infection by genetically distinct SARS-CoV or animal strains of SARS-CoV-like viruses?
- How long does protection induced by vaccines last?
- Are there *in vivo* correlates of enhanced entry mediated by antibodies to certain SARS-CoV-like antibodies?

Acknowledgements

This research was partly supported by the Intramural Research Program of the NIH, NIAID.

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